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Multiresidue Analysis of Organochlorines, Synthetic Pyrethroids and Organophosphorous Pesticides in Pulse Grain Samples

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A multiresidue method was developed and validated for simultaneous determination of 11 organochlorines, 8 synthetic pyrethroids and 14 organophosphorous pesticides in green gram and black gram grain samples using gas chromatography - electron capture detector and flame thermoionic detector (GC-ECD/FTD). The QuEChERS principle based methodology was adopted for extraction. Samples were extracted with acetonitrile and the co-extractives were removed using dispersive solid-phase extraction (dispersive-SPE) with primary secondary amine (PSA) after salting out with NaCl and $MgSO_4$. Average recoveries for all the pesticides studied were between 71.70 and 113.31 % and relative standard deviation (RSD) ranged between 0.10 and 12.79 %. The present study indicates that the proposed method is useful in monitoring Organic Chlorine, Synthetic Pyrethroids and Organo Phosphorus insecticides in selected pulse grains.

Key words: Green gram, black gram, multiresidue analysis, QuEChERS

Grain legumes such as chickpea, pigeon pea, cowpea, black gram and green gram play an important role in food and nutritional security apart from sustainable crop production (Sharma *et al*., 2010). Green gram (*Vigna radiata* L.) and black gram (*Vigna mungo* L.) are the most important and widely cultivated pulse crops in India. The pulse crops are infested by a large number of insect pests, such as pod borers, aphids, jassids and pod ûies, which results in reduction of crop yield. Among these pests, pod borer complex cause major yield loss (Mukherjee *et al.,* 2007). Management of insect pests in pulses relies heavily on insecticides often to the exclusion of other methods. Pesticides like lindane and fenvalerate (Parihar and Gupta, 2001; Madan *et al*., 2000); dimethoate; beta-cyfluthrin (Mukherjee *et al*., 2007) and endosulfan (Chowdhury *et al*., 2007; Singh *et al*., 2009) were used for the control of insect pests in various pulse crops.

Even though use of such insecticides aid in controlling of insect pests and increase productivity, they have residual effect. The persistence of small quantity of pesticides can cause health hazards to human beings. Therefore it is necessary to identify, quantify and monitor pesticide residues in whole pulses as well as in its product.

A number of sample preparation techniques and method of analyses have been developed for the pesticide residue determination in a wide range of foodstuffs and other agricultural products (Cieslik *et al*., 2011). Conventional methods of analysis involve complex extraction techniques *viz*., liquid-liquid extraction and Soxhlet extraction (SPE cleanup) followed by analysis in gas chromatography using a variety of detectors (Cieslik *et al*., 2011). All these methods are complex, laborious, time consuming, expensive, require large amounts of organic solvents and usually involve many steps, leading to the loss of analyte quantity (Durovicc and Dordevic, 2011).

The QuEChERS approach (Quick, Easy, Cheap, Effective, Rugged and Safe) was introduced by Anastassiades *et al*. (2003) and become the method of choice for the rapid extraction and clean up of various types of samples to determine pesticide residues followed by gas chromatography (GC) analysis (Curbelo *et al.,* 2012). In QuEChERS method, the analyte from the substrate is extracted with acetonitrile followed by removal of water from the extract by salting out with sodium chloride and magnesium sulphate. The extract is subjected to further cleanup using dispersive-solid phase extraction (SPE) technique, which involves PSA sorbent and $MgSO_4$. The advantages of QuEChERS over the other methods are high recoveries for wide range of polarity and volatility of pesticides, high accuracy, high sample throughput, small solvent usage and wastage, simplicity, use of simple laboratory apparatus, little bench space and minimal solvent exposure to workers (Chai *et al*., 2012).

In the present study, multiresidue method was developed and validated for simultaneous determination of 14 organophosphorous (OP), 11

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organochlorines (OC) and 8 synthetic pyrethroids (SP) pesticide residues in pulse grains.

Materials and Methods

Chemicals and reagents

Certified reference standards of 11 organochlorine (á- HCH, â-HCH, ã-HCH, ä-HCH, dicofol, pp -DDT, pp-DDD, pp-DDE, alpha endosulfan, beta endouslfan, and endosulfan sulphate), 14 organophosphorous (dichlorvos, acephate, monocrotophos, phorate, dimethoate, phosphamidon, parathion methyl, fenitrothion, malathion, chlorpyriphos, quinalphos, profenophos, ethion and triazophos) and 8 synthetic pyrethroids (bifenthrin, fenpropathrin, lambda cyhalothrin, alpha cypermethrin, beta cyfluthrin, fenvalerate, fluvalinate and deltamethrin) pesticides were obtained from M/s Sigma Aldrich, Germany with purity ranging from 95.1 to 99.9%.

Residue analytical grade acetone, acetonitrile and hexane (HPLC grade), anhydrous magnesium sulphate and sodium chloride were purchased from M/s. Merck (Mumbai, India), primary secondary amine (Bondesil PSA, 40 µm particle size) was obtained from M/s. Varian, India.

Preparation of standards

Stock solutions (1000 µg ml^{-1}) of each pesticide standard were prepared by dissolving 0.025 g of the pesticide in 25 ml of hexane/acetone (9/1, v/v) mixture. An intermediate stock standard mixture of 10 µg ml^{-1} was prepared by mixing appropriate quantities of the individual stock solutions and diluting accordingly. Working standard solutions of 0.01- 0.1µg ml^{-1} were prepared and injected to obtain the linearity of detector response. All the stock and working standard solutions were stored in the dark at - 4 ºC until further use.

Sample preparation

Green gram and black gram grain samples were collected from the local market in Coimbatore (Tamil Nadu). Samples were ground using high volume blade homogenizer and used for the present study.

Extraction and cleanup

Extraction and clean -up was carried out according to QuEChERS method as originally described by Anastassiades *et al*. (2003) with some minor modifications. The entire pulse grain sample was fine ground and ten gram of representative sample was weighed in a 50 ml screw-capped centrifuge tube and 20 ml of acetonitrile was added, screw cap was closed and the tube was vigorously shaken by hand for one min. Afterwards, to induce phase separation and pesticide partitioning, salt mixture consisting of 4 g of anhydrous magnesium sulfate and 1 g of sodium chloride was added and then each tube was shaken vigorously by hand for 2 min to prevent precipitation of magnesium sulfate. After vigorous shaking, tubes were placed in a centrifuge (Plasto crafts, Mumbai) and centrifuged at 5000 rpm for 10 min at room temperature.

For clean-up a 6 ml aliquot of the extract (upper organic phase) was transferred into a 15 ml polypropylene tube containing 100 mg primary secondary amine (PSA) and 600 mg anhydrous $MgSO_4$. The tube was closed, then shaken for 30 seconds using a vortex mixer and was then centrifuged for 5 min at 3000 rpm. Finally, 4 ml of the final extracts was evaporated under a gentle stream of nitrogen (15 psi) by using the Turbovap LV (Caliper Life Sciences, Russelsheim, Germany) set at a temperature of 40 °C, until near dryness. The residues were then redissolved in 1.0 ml of hexane and the extract was transferred to an autosampler vial and analyzed using GC-ECD and FTD.

Recovery study

The recovery study was conducted by spiking known quantity of pesticide standard on to the sample at three different levels (10, 50, and 100 ng g^{-1}) and with three replications each. In control sample, same amount of hexane was added. The spiked samples were mixed well and left for 15 min (equilibration time) at room temperature prior to extraction. The extraction process was done according to the procedure described above. Repeatability of the method was evaluated through the relative standard deviation (RSD %).

Instrumental parameters

GC-ECD/FTD chromatographic conditions used for the determination of selected organochlorines, synthetic pyrethroids and organophosphorous pesticides are described below.

GC-ECD analysis: The quantification of OC and SP was performed using GC (Shimadzu GC-2010) equipped with an EC detector, an auto sampler (AOC 20s) and an auto injector (AOC 20i). The OC and SP was separated using DB-5 (30m x 0.25 mm ID, 0.25 micron film thickness) fused silica capillary column (J & W Scientific Co., Agilent Technologies, and USA). The injection was performed at split (1:10) mode with 1 µl injection volume. High purity (purity 99.999 %) nitrogen was used as carrier and makeup gas flow at 2 ml min^{-1} and 30 ml min^{-1}, respectively. The injector temperature was 280°C and the detector temperature was 300 °C. The oven temperature was programmed as follows: 160°C, for 1 min, increased to 200°C (3°C min^{-1}) and held for 2 min, increased to 220°C (4°C min^{-1}) and maintained for 4 min and finally, increased to 250 °C (5 °C min^{-1}) and kept for 18 min.

GC-FTD analysis: The quantification of OPs was performed using GC (Shimadzu GC-2010) equipped with FTD detector, an auto sampler (AOC 20s) and an auto injector (AOC 20i). The gas chromatographic separations were performed using a DB-1 MS fused

silica capillary column, 30 m x 0.25 mm ID, 0.25 micron film thickness (J & W Scientific Co., Agilent Technologies, USA) with maximum temperature of 325°C. The carrier gas was helium, with a flow of 1.0 ml min^{-1} and a column head pressure of 186.4 kPa. Flow relation for the FTD detector was 2 ml min^{-1} for hydrogen and 145 ml min^{-1} for air. One ìl sample was injected into the GC instrument using the split mode (1:5) with linear velocity flow control mode. The injector and detector were operated at 280 and 300°C, respectively. The column temperature was initially set at 160°C for 1 min, increased to 200°C at the rate of 3°C min^{-1} and held for 2 min, increased to 220°C at the rate of 4°C min^{-1} and held for 4 min and finally, increased to 250°C, at the rate of 5 and held for 18 min. The total analysis time was 54.33 min and the equilibrium time was 2 min.

Results and Discussion

Extraction and cleanup

For extraction and cleanup, QuEChERS method (Anastassiades *et al*., 2003) was followed with slight modifications. Initially, this method was used for the extraction of pesticide residues in fruits and vegetables later, it was applied for wide range of food matrices.

In the present study, pesticides were extracted from spiked as well as control black gram and green gram grain samples by mixing the extracts with acetonitrile followed by removal of water from the extract by salting out with sodium chloride and magnesium sulphate. The purpose of adding acetonitrile in our method was due to its high selectivity (few co-extractives), easily miscible with water and separates from the water phase with the

Table 1. Analytical recovery of organochlorine pesticides in green gram grain samples determined using GC-ECD

Pesticide	Fortification levels						
		10 ng g^{-1}		50 ng g^{-1}		100 ng g^{-1}	
	Retention time (min)	Mean Recovery (%)	Precision (RSD) (%)	Mean Recovery (%)	Precision (RSD) (%)	Mean Recovery (%)	Precision (RSD) (%)
α- HCH	7.067	76.00	3.71	71.70	1.82	85.64	2.14
β- HCH	8.018	92.27	2.00	81.62	1.59	86.59	1.98
γ- HCH	8.237	76.22	0.81	77.31	1.81	91.02	0.97
δ- HCH	9.148	83.75	2.23	75.22	1.27	87.31	1.54
Dicofol	13.316	86.76	8.25	94.03	0.79	93.05	3.34
α- Endosulfan	16.852	75.87	0.10	77.40	1.78	82.00	1.22
p,p' DDE	18.539	93.56	3.74	95.14	2.50	93.87	0.17
β- Endosulfan	20.132	86.91	8.67	82.14	0.80	85.79	1.10
p,p' DDD	20.901	85.63	4.56	83.06	2.16	91.47	1.82
Endosulfan sulphate	22.585	93.96	1.12	78.63	1.63	80.71	0.71
p,p' DDT	23.074	89.25	3.90	82.31	0.87	88.91	1.41

Table 2. Analytical recovery of organophosphorous pesticides in green gram grain samples determined using GC-FTD

Pesticide	Fortification levels						
		10 ng g^{-1}		50 ng g^{-1}		100 ng g^{-1}	
	Retention time (min)	Mean Recovery (%)	Precision (RSD) (%)	Mean Recovery (%)	Precision (RSD) (%)	Mean Recovery (%)	Precision (RSD) (%)
Dichlorvos	2.99	94.69	8.65	87.64	12.79	86.09	6.11
Acephate	4.119	78.74	7.23	80.92	7.60	81.97	2.20
Monocrotophos	7.225	90.64	4.19	76.36	5.86	93.96	4.90
Phorate	8.144	95.92	2.39	81.99	2.02	91.04	0.87
Dimethoate	8.310	91.21	6.19	84.53	2.82	99.37	1.36
Phosphamidon (1+2)	10.061						
	11.615	96.10	4.39	93.42	1.23	99.65	1.67
Methyl parathion	12.016	91.09	3.99	93.37	1.30	93.46	1.56
Fenitrothion	13.349	96.47	1.68	97.48	0.45	96.87	1.46
Malathion	14.017	94.17	0.54	91.15	2.54	92.11	3.70
Chlorpyriphos	14.694	91.47	1.92	92.19	1.50	90.27	4.87
Quinalphos	17.030	100.67	2.74	97.84	1.13	97.49	1.80
Profenophos	19.723	101.39	1.74	98.73	3.72	97.04	2.35
Ethion	22.605	113.31	4.36	86.18	3.39	91.22	2.10
Triazophos	22.685	91.21	4.61	96.08	4.02	94.34	7.12

addition of salt. Cleanup was performed by using primary secondary amine (PSA) solid phase extraction (SPE), which involved mixing the extract in a mixer with PSA and $MgSO_4$. The dispersive-SPE with PSA effectively removes many polar matrix components, such as organic acids, certain polar pigments and sugars to some extent from the food extracts (Anastassiades *et al* ., 2003). SPE is a multifunctional technique, since the purification and the concentration occurs in the same step (Durovicc and Dordevic, 2011).

Recovery

Table 1, 2 and 3 summarize the average recovery and relative standard deviations (RSD) of the pesticides studied. Spiked green gram samples gave satisfactory recovery rates for the target analytes (OCs and OPs). Recoveries obtained in green gram for OCs at the level of 10, 50 and 100 ng g^{-1} were 75.87 to 93.96 %, 71.70 to 95.14% and 80.71 to 93.87 %, respectively with RSD of 0.10 to 8.67% (Table 1). Similarly, recoveries obtained for OP at

Table 3. Analytical recovery of synthetic pyrethroid pesticides in black gram grain samples using GC-ECD

Pesticide	Fortification levels						
		10 ng g^{-1}		50 ng g^{-1}		100 ng g^{-1}	
	Retention time (min)	Mean Recovery (%)	Precision (RSD) (%)	Mean Recovery (%)	Precision (RSD) (%)	Mean Recovery (%)	Precision (RSD) (%)
Bifenthrin	27.436	92.81	0.97	99.94	4.15	97.75	1.97
Fenpropathrin	27.717	80.90	5.36	79.85	5.39	106.28	1.04
λ- cyhalothrin	30.749	82.07	6.78	98.16	2.44	94.55	3.86
α- Cypermethrin	36.950	72.41	9.42	89.60	3.93	84.54	1.42
β- Cyfluthrin (1+2+3+4)	35.394						
	35.667						
	35.865						
	36.126	111.11	4.48	107.22	3.27	104.14	1.82
Fenvalerate (1+2)	40.573						
	41.864	77.37	8.49	71.90	3.15	83.97	0.83
Fluvalinate(1+2)	42.187						
	42.739	73.15	0.73	76.13	0.00	78.63	1.11
Deltamethrin	45.541	105.59	5.04	110.52	3.62	98.75	5.47

10, 50 and 100 ng g^{-1} concentrations were 78.74-113.31 %, 76.36-98.73 % and 81.97-99.65 %, respectively with RSD of 0.45-12.79% (Table 2).

The mean recoveries of Synthetic Pyrethroids compounds were satisfactory and in the range of 71.90 % to 111.11 %, with good RSD values lower than 9.42% (Table 3).

Experimental data demonstrate that the recovery and reproducibility obtained by the proposed multiresidue method was satisfactory. The average range of recovery of the pesticides determined by the proposed method fall within the acceptable recovery ranges of 70-120% with RSD of less than 20% follows the recent European Union guidelines (SANCO, 2009).

Results obtained in the present study are similar to the one reported by Chai *et al*. (2012) who extracted the green yardlong beans spiked with 20 pesticides by QuEChERS method using dispersive SPE cleanup and analysed by gas chromatography with Flame photometric and Electron capture detectors and similar recoveries (87-112%) have been reported. These results demonstrate that the method has good reproducibility and the recovery of pesticides was satisfactory.

Conclusion

The modified QuEChERS method was evaluated for the measurement of pesticide residues in black gram and green gram grain samples. The method proved to be fast, easy to conduct, and used only small quantities of solvents, glasswares and reagents. The results show good analytical performance in terms of good repeatability and recovery in almost all pesticides studied. The proposed method can be used as a monitoring technique to determine residue levels in commercial pulse grain samples.

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